DATA EVALUATION RECORD

FLUTRIAFOL (PP450)

Study Type: OPPTS 870.3700a [§83-3a]; Developmental Toxicity Study in Rats

Work Assignment No. 5-01-151 D; formerly 4-01-151 D (MRID 47090349)

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Template version 02/06

TXR #: 0054780

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study – Rat (gavage); OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 128940 **DP BARCODE:** 340368

TEST MATERIAL (PURITY): Flutriafol Technical (93% a.i.)

SYNONYMS: PP450; α-(2-fluorophenyl)-α-(4-fluorophenyl)-1*H*-1,2,4-triazole-1-ethanol

CITATION: PP450 (Flutriafol): Teratogencity study in the rat. Imperial Chemical Industries PLC, Cheshire, UK. Laboratory Study No. RR0211, Report No. CTL/P/756, October 27, 1982. MRID 47090349. Unpublished.

SUBMITTER/SPONSOR: Cheminova Inc., 1600 Wilson Boulevard, Suite 700, Arlington, VA (originally sponsored by Imperial Chemical Industries PLC)

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 47090349), Flutriafol (PP450; 93%; Batch # P10) in corn oil was administered via daily oral gavage in a dose volume of 10 mL/kg to 24 presumed pregnant Wistar rats at doses of 0, 10, 50, or 125 mg/kg/day from gestation days (GD) 6-15. On GD 21, all dams were euthanized; each dam's uterus was removed via cesarean section and its contents examined. Fetuses were examined for external, visceral, and skeletal malformations and variations.

All dams survived until scheduled termination. There were no treatment-related macroscopic findings.

Increased incidence of staining of the genital/ventral fur was observed primarily during the dosing period in 16 dams at 125 mg/kg/day compared to 7 dams in the control group. Additionally at 125 mg/kg/day, maternal body weight gains were decreased ($p<=0.01$) during the treatment (decr. 26%) and post-treatment (decr. 33%) intervals, and for the overall study (decr. 23%). Overall net weight gain, corrected for gravid uterine weight, was decreased by 19% compared to controls. Food consumption was decreased by 14-17% at this dose compared to controls during the treatment and post-treatment intervals.

The maternal LOAEL is 125 mg/kg bw/day based on increased incidence of ventral/genital staining of the fur and decreased maternal body weight gains and food consumption. The

maternal NOAEL is 50 mg/kg bw/day.

The number and percent of early intrauterine deaths were increased at 125 mg/kg/day (40 deaths; 14.8%) compared to controls (15 deaths; 6.3%), with a significantly higher ($p<=0.05$) proportion of dams affected at 125 mg/kg/day (14/21 dams) compared to controls (6/20 dams). Similarly, the number and percent of late intrauterine deaths were increased at 125 mg/kg/day (46 deaths; 18.7%) compared to controls (0 deaths; 0%), with a significantly higher ($p<=0.05$) proportion of dams affected at 125 mg/kg/day (14/21 dams) compared to controls (0/20 dams). The increases in early and late intrauterine deaths were reflected by an increased post-implantation loss at this dose (33.5% affecting 17/21 dams) compared to controls (6.3% affecting 6/20 dams).

Additionally at 125 mg/kg/day, mean gravid uterine weight, total litter weight, and live fetal body weights were decreased ($p<=0.01$) by 16-27%.

Incidences of the following skeletal variations, indicating skeletal retardation, were increased ($p<=0.05$) over concurrent controls and/or the provided historical control data: (i) in all treated groups - incompletely ossified unilateral and/or bilateral calcanea, partially ossified occipital, and not ossified odontoid; (ii) in the 50 and 125 mg/kg/day fetuses - unilateral and/or bilateral cervical rib and unilateral and/or bilateral extra (14) ribs; (iii) at 125 mg/kg/day - partially ossified parietals, increased fontanelle, partially ossified cervical arches between and including #3 and #6, partially ossified 1st sternebra, partially ossified 2nd sternebra, not ossified 5th sternebra, partially ossified, not ossified 6th sternebra, and partially ossified frontals.
Aside from the variations listed above indicating skeletal retardation, there were no treatment-related external, visceral, or skeletal variations.

There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL is 50 mg/kg bw/day based on delayed ossification or non-ossification of the skeleton in the fetuses. The developmental NOAEL is 10 mg/kg bw/day.

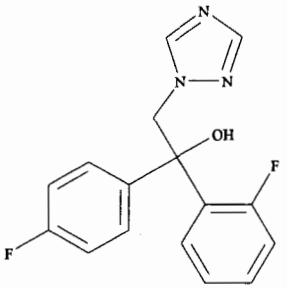
This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Flutriafol (PP450) Technical

Description:	White solid
Batch #:	P10
Purity:	93%
Compound stability:	The test substance was stable in the vehicle for at least 10 days (room temperature assumed) based upon concentration analyses conducted prior to, and at the end of, the dosing period.
CAS #:	76674-21-0
Structure:	

2. Vehicle: Corn oil

3. Test animals

Species:	Rat
Strain:	Specific-Pathogen-Free, Wistar-derived
Age/weight at study initiation:	Approximately 11-13 weeks; 214-291 g
Source:	Alderley Park, Cheshire, UK
Housing:	Individually in suspended polypropylene cages with stainless steel wire mesh floors
Diet:	Pelleted Portion Combined Diet (PCD), Special Diet Services Ltd., Stepfield, Witham, Essex, UK, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-22°C **Humidity:** 40-64% **Air changes:** ≥10/hr **Photoperiod:** 12 hours light/12 hours dark
Acclimation period:	None (Animals were received at the performing laboratory on GD 0)

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: March 9, 1982 End: April 2, 1982

2. Mating: Adult nulliparous female rats were paired with males of the same strain overnight at the supplier's breeding unit. The morning following pairing, female rats were examined for positive evidence of mating, as indicated by the presence of spermatozoa in a vaginal smear. The day on which positive evidence of mating was found was designated as gestation day (GD) 0. Successfully mated females were delivered to the performing laboratory on GD 0. Twenty four females were supplied on each of four consecutive days.

3. **Animal assignment:** Mated females were randomly assigned to the dose groups shown in Table 1.

TABLE 1. Animal Assignment [a]				
Dose (mg/kg/day)	0	10	50	125
No. females	24	24	24	24

a Data were obtained from page 13 of the study report.

4. **Dose selection rationale:** The dose levels were selected based on the results from two preliminary studies conducted to determine the maximum tolerated dose to non-pregnant rats and the effects on pregnant female rats and their developing fetuses. It was stated that slight reductions in maternal body weight gains and increased post-implantation loss were observed at 100 mg/kg/day; and maternal toxicity, embryolethality, and decreased fetal body weights were observed at 150 mg/kg/day. No further information was provided.

5. **Dose preparation, administration, and analysis:** Dose formulations were administered via oral gavage in a dose volume of 10 mL/kg daily from gestation days (GD) 6-15. Dose formulations were prepared once prior to the start of the dosing period. For each dose level, a weighed amount of the test material (adjusted for purity) was suspended in an appropriate volume of corn oil to achieve the desired concentration. The suspensions were ball-milled for approximately 24 hours. Following preparation, concentration analyses were performed on samples of each concentration (including the corn oil control); and homogeneity of the 50 and 125 mg/kg/day doses was verified. Stability of the test material in corn oil was determined at each concentration at the end of the dosing period. Dose suspensions were stirred with a magnetic stirrer prior to administration or analyses. Storage conditions were not reported.

Results

Concentration (mean % nominal pre-dosing): 89-101.6%

Stability (mean % nominal post-dosing): 93-124%

Homogeneity: 97.6-102% nominal; 1.15-1.68% coefficient of variation

The analytical data indicated that the mixing procedure was adequate and the variation between the nominal and actual dosage to the animals was acceptable

C. OBSERVATIONS

1. **Maternal observations and evaluations:** External physical examinations were conducted on all female rats upon arrival at the performing laboratory to ensure that they were physically normal. All animals were checked daily throughout the study for mortality and

clinical signs of toxicity. Body weights were recorded on GD 0, 6-15 (inclusive), and 21. Body weight gains were reported for GD 0-6 (pre-treatment), 6-15 (treatment), 15-21 (post-treatment), and 0-21 (overall study). Additionally, net overall body weight gain (corrected for gravid uterine weight) was calculated by the reviewers by subtracting the weight of the gravid uterus from the body weight gain for GD 0-21. Food consumption (g/rat/day) was determined for the pre-treatment (GD 0-6), treatment (6-15), and post-treatment (15-21) intervals. On GD 21, the dams were euthanized with an overdose of halothane BP vapor via inhalation and were subjected to a gross necropsy. A cesarean section was performed, and the gravid uterus was removed and weighed. The number of corpora lutea in each ovary was counted. The uterine contents were then examined, and the number and position of implantations were recorded. Implantations were categorized as live fetuses, early intrauterine deaths, or late intrauterine deaths. Early intrauterine deaths were defined as showing decidual or placental tissue only, whereas late intrauterine deaths were characterized by embryonic or fetal tissue in addition to placental tissue.

2. **Fetal evaluations:** All live fetuses were weighed and then killed by an intracardiac injection of Euthatal (Pentobarbitone Sodium solution) and examined for external abnormalities. Approximately two thirds of the fetuses from each litter were randomly selected, examined for visceral abnormalities, and sexed. These fetuses were then eviscerated and fixed in 70% methanol prior to processing and staining with Alizarin Red S using the method of Staples and Schnell (1964). The stained fetal skeletons were examined for abnormalities, and the degree of ossification was assessed. The individual bones of the hand (*manus*) and foot (*pes*) were assessed, and the result converted to a semi-quantitative four point scale found in Appendix 4 on page 45 of the study report, included as an Attachment to this DER. The remaining fetuses were fixed and decalcified in Bouin's solution for at least 10 days, and then stored in 70% methanol for subsequent soft tissue examination. The head of each fetus was serially sectioned using the technique of Wilson (1965). The thoracic and abdominal cavities were examined by dissection, and the sex of each fetus was recorded. The kidneys were sectioned transversely to examine their internal structures.

D. DATA ANALYSIS

1. **Statistical analyses:** Data from animals which were not pregnant or had no live fetuses were excluded from statistical analyses. The analysis of variance (ANOVA) allowed for the replicate design of the study and the day of arrival of the animals. Individual group means were adjusted for missing values prior to pair-wise comparison of the treated groups with the controls using Student's t-test. All statistical tests were one-sided, with the following exceptions which were two-sided: body weight gain; food consumption, the number of corpora lutea; and the proportion of male fetuses. Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. It was not stated whether the assumptions of homogenous variances and normal distribution of the data were tested prior to proceeding with parametric analyses. Otherwise, the statistical analyses were considered appropriate.

Parameter	Statistical test
Initial (GD 0) maternal body weight Maternal body weight gains for GD 0-6, 6-15 and 15-21 Maternal food consumption for GD 0-6, 6-15 and 15-21 Numbers of corpora lutea, implantations, and live fetuses per dam Gravid uterine weight, total litter weight, and mean fetal weight (calculated on an individual litter basis) Mean *maus* and *pes* scores per fetus	Analysis of variance (ANOVA) followed by pair-wise comparison of the treated groups with the controls using Student's t-test
Percent pre-implantation loss and post-implantation loss Percent fetuses with minor external, visceral, or skeletal defects (calculated on an individual litter basis)	Double arc-sine transformation of Freeman and Tukey (1950) prior to ANOVA. Student's t-test was conducted for pair-wise comparison of the treated groups with the controls.
Proportions of females with pre-implantation loss, post-implantation loss, early intra-uterine deaths, and late intrauterine deaths Proportion of male fetuses Proportion of fetuses with minor external, visceral, or skeletal defects, and other specific skeletal findings (calculated on an individual litter basis)	Fisher's Exact Test for pair-wise comparison of treated groups with the controls

2. **Indices:** The following indices were reported:

   Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea x 100

   Post-implantation loss (%) = (# implantations – # live fetuses)/ # implantations x 100

   For pre-implantation loss, the difference between the number of corpora lutea and the number of implantations was assumed to be zero for females for which the corpora lutea count was exceeded by the number of implantations.

3. **Historical control data:** Historical control data from developmental toxicity studies conducted at the performing laboratory using the same strain of rat were provided. These data comprised incidences of skeletal malformations, variations, retardations, and mean *manus* and *pes* scores from 15 studies conducted from 1977-1984.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations

a. Mortality: All dams survived until scheduled termination.

b. **Clinical signs of toxicity:** Staining of the genital/ventral fur primarily during the dosing period was found in the control (7 dams), 10 mg/kg/day (8 dams), 50 mg/kg/day (4 dams) and 125 mg/kg/day (16 dams) groups. The increased incidence at 125 mg/kg/day is considered treatment-related. No other summary data were provided. However, it was stated that other clinical observations, such as fur loss, were of low incidence in all groups including the control and were not considered treatment-related.

2. **Body weight:** During the pre-treatment interval, body weight gains in all treated groups were comparable to controls (Table 2). At 125 mg/kg/day, maternal body weight gains were decreased ($p \leq 0.01$) during the treatment (↓26%) and post-treatment (↓33%) intervals, and for the overall study (↓23). Gravid uterine weights were decreased ($p \leq 0.01$) by 27% at this dose, and the overall net weight gain corrected for gravid uterine weight (calculated by the reviewers) was decreased by 19% compared to controls. Body weight gains in the 10 and 50 mg/kg/day dams were comparable to controls throughout the study.

TABLE 2. Mean maternal initial body weight and body weight gains (g) [a]

Interval		Dose in mg/kg/day [# dams] [b]			
		0 [20]	10 [21]	50 [23]	125 [21]
Initial body weight	GD 0	255.3	251.5	255.2	241.1
Pre-treatment	GD 0-6	29.1	31.1	30.8	31.7
Treatment	GD 6-15	41.6	43.6	41.4	30.8** (↓26)
Post-treatment	GD 15-21	89.2	86.1	85.4	60.2** (↓33)
Overall gain	GD 0-21	160.0	160.8	157.6	122.7** (↓23)
Gravid uterine weight [c]		84.4	81.7	79.2	61.5** (↓27)
Corrected weight gain (GD 0-21) [d]		75.6	79.1	78.4	61.2 (↓19)

a Data were obtained from Table 4 on page 26 and Table 6 on page 30 of the study report. Percent difference from controls, calculated by the reviewers, is included in parentheses. Standard deviations were not provided.

b n = number of dams with live fetuses.

c The mean gravid uterine weights were based on an n = 18, 18, 20, and 19 in the control, 10, 50, and 125 mg/kg/day groups, respectively. These values exclude 1 gravid uterus weight which was not recorded and 9 which were recorded incorrectly.

d Calculated by the reviewers by subtracting the mean gravid uterine weight from the mean overall (GD 0-21) gain. Note that this calculation is an approximation, because 2 to 3 gravid uterine weight values per group were excluded.

** Significantly different from the control group at $p \leq 0.01$

3. **Food consumption:** During the pre-treatment interval, maternal food consumption in all treated groups was comparable to controls (Table 3). At 125 mg/kg/day, food consumption was decreased by 14-17% compared to controls during the treatment and post-treatment intervals. Food consumption in the 10 and 50 mg/kg/day dams was comparable to controls throughout the study.

TABLE 3. Mean maternal food consumption (g/animal/day) [a]					
Interval		Dose in mg/kg/day [# dams] [b]			
		0 [20]	10 [21]	50 [23]	125 [21]
Pre-treatment	GD 0-6	24.2	24.5	24.6	24.8
Treatment	GD 6-15	22.6	22.4	22.2	18.8** (↓17)
Post-treatment	GD 15-21	35.5	35.0	35.4	30.6** (↓14)

a Data were obtained from Table 5 on page 27 of the study report. Percent difference from controls, calculated by the reviewers, is included in parentheses. Standard deviations were not provided.

b Number of dams with live fetuses.

** Significantly different from the control group at $p \leq 0.01$

4. **Gross pathology:** It was stated that no treatment-related macroscopic findings were observed at necropsy. However, no data were provided.

5. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 4. The number and percent of early intrauterine deaths were increased at 125 mg/kg/day (40 deaths; 14.8%) compared to controls (15 deaths; 6.3%), with a significantly higher ($p \leq 0.05$) proportion of dams affected at 125 mg/kg/day (14/21 dams) compared to controls (6/20 dams). Similarly, the number and percent of late intrauterine deaths were increased at 125 mg/kg/day (46 deaths; 18.7%) compared to controls (0 deaths), with a significantly higher ($p \leq 0.01$) proportion of dams affected at 125 mg/kg/day (14/21 dams) compared to controls (0/20 dams). The increases in early and late intrauterine deaths were reflected by an increased ($p \leq 0.01$) post-implantation loss at this dose (33.5% affecting 17/21 dams) compared to controls (6.3% affecting 6/20 dams). The number of dams with live fetuses was reported, but no explanation was provided for the difference between the number of females mated and the number of females with live fetuses. Thus, the reviewers were unable to determine if the number of animals without live fetuses in each group was due to non-pregnancy, abortion, premature delivery, or complete litter resorption. Because no mention was made of abortion, premature delivery, or complete litter resorption, it is likely that these animals were not pregnant. However, the number of females with live litters was comparable among treated and control groups. The number of live fetuses/dam was decreased (↓31) in the 125 mg/kg/day group compared to controls. Additionally at 125 mg/kg/day, decreases ($p \leq 0.01$) in mean gravid uterine weight (↓27%), total litter weight (↓39%), and live fetal body weights (↓16%) were observed.

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TABLE 4. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	10	50	125
No. Animals assigned (mated)	24	24	24	24
No. Animals with live fetuses	20	21	23	21
No. Animals without live fetuses [b, c]	4	3	1	3
Maternal wastage				
No. died	0	0	0	0
No. Died pregnant	0	0	0	0
No. Died nonpregnant	0	0	0	0
No. Aborted [c]	NR	NR	NR	NR
No. Premature deliveries [c]	NR	NR	NR	NR
Mean No. Corpora lutea	15.0	14.4	13.8	14.0
Mean No. Implantations	12.9	12.6	11.8	12.4
Total No. litters	20	21	23	21
Total No. live fetuses	237	247	256	171
Mean No. Live fetuses	12.2	11.7	11.1	8.4** (↓31)
Early intra-uterine deaths (No.)	15	19	16	40
Mean percent (%)	6.3	7.2	6.1	14.8
Proportion of dams affected	6/20	13/21*	10/23	14/21*
Late intra-uterine deaths	0	2	1	46
Mean percent (%)	0	0.7	0.4	18.7
Proportion of dams affected	0/20	2/21	1/23	14/21**
Complete litter resorptions [c]	NR	NR	NR	NR
Mean gravid uterus weight (g)	84.4	81.7	79.2	61.5** (↓27)
Mean total litter weight	63.9	59.9	57.8	38.7** (↓39)
Mean live fetal weight (g)	5.27	5.09	5.24	4.44** (↓16)
Sex ratio (% male)	49	46	52	47
Pre-implantation loss (%) [d]	13.5	10.3	13.8	12.2
Proportion of dams affected	15/20	10/21	12/23	13/21
Post-implantation loss (%) [e]	6.3	7.9	6.4	33.5** [f]
Proportion of dams affected	6/20	13/21*	10/23	17/21**

a Data were obtained from Table 6 on pages 28-30 in the study report. Percent difference from the control group, calculated by the reviewers, is included in parentheses. Standard deviations were not provided.

b Calculated by the reviewers from data presented in this table.

c The reviewers were unable to determine if the number of animals without live fetuses in each group was due to non-pregnancy, abortion, premature delivery, or complete litter resorption.

d Pre-implantation loss (%) = (mean # corpora lutea – mean # implantations)/mean # corpora lutea x 100

e Post-implantation loss (%) = (mean # implantations – mean # live fetuses)/mean # implantations x 100

f Although statistics were performed on the double arc-sine transformed values, the reviewers denoted significance next to the untransformed data presented in this table.

NR Not reported

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the controls at $p \leq 0.01$

B. DEVELOPMENTAL TOXICITY

1. **External examination:** All external findings are presented in Table 5. Dark red area on the skull was observed in one fetus at 10 mg/kg/day, and subcutaneous hemorrhage was noted in one fetus at 50 mg/kg/day and one fetus at 125 mg/kg/day. However, these findings were considered unrelated to treatment because they only occurred in a maximum of a single fetus per group for each finding and/or because they were unrelated to dose. No other external findings were noted.

TABLE 5. External findings (# fetuses affected) [a]

Observation	Dose (mg/kg/day)			
	0	10	50	125
No. Fetuses (litters) examined	237 (20)	247 (21)	256 (23)	171 (21)
Variations (minor defects)				
Dark red area on skull	---	1	---	---
Subcutaneous hemorrhage	---	---	1	1

a Data were obtained from Tables 7 and 8 on pages 31-32 of the study report.
--- No animals affected (i.e., zero incidence)

2. **Visceral examination:** Selected visceral findings are presented in Table 6. Microphthalmia was observed in a single 10 mg/kg/day fetus, and unilateral agenesis of the kidney and adrenal gland was noted in a single 125 mg/kg/day fetus. These malformations (referred to in the study report as major defects) were considered unrelated to treatment because they were only observed in a single fetus per finding. No other visceral malformations were observed. At 125 mg/kg/day, abdominal ascites was observed in 2 fetuses, and mesenteric hemorrhage was noted in 1 fetus. These variations (referred to in the study report as minor defects and/or variants) were considered unrelated to treatment because they were minimal in incidence. All of the other visceral variations were unrelated to dose.

TABLE 6. Selected visceral findings (# fetuses affected) affected [a]

Observation	Dose (mg/kg/day)			
	0	10	50	125
No. Fetuses (litters) examined	237 (20)	242 (21)	246 (23)	171 (21)
Malformations (major defects)				
Eyes - microphthalmia	---	1	---	---
Urogenital – unilateral agenesis of kidney and adrenal	---	---	---	1
Variations (minor defects)				
Abdomen – abdominal ascites	---	---	---	2
mesenteric hemorrhage	---	---	---	1

a Data were obtained from Tables 7 and 8 on pages 31-32 of the study report.
--- No animals affected (i.e., zero incidence)

3. **Skeletal examination:** Incidences of selected skeletal findings are presented in Table 7a. Kinky ribs were observed in two fetuses (1.8%) in the 125 mg/kg/day group compared to 0 controls. No historical control data were presented for this finding. Because this finding was only noted in two fetuses, it is unlikely that it is due to treatment. A single fetus at 50 mg/kg/day had multiple malformations, including 7th cervical vertebral arches missing, 4th cervical arch missing on the right side, all cervical centra present but misaligned, 5th thoracic arch and 5th rib on the right side missing, and 3rd and 4th thoracic arches slightly misaligned. There were no other skeletal malformations.

   Incidences of the following skeletal variations, indicating skeletal retardation, were increased ($p \leq 0.05$) over concurrent controls and/or the historical control data provided:
   (i) incompletely ossified unilateral and/or bilateral calcanea in all treated groups (82.5-100%) compared to concurrent (70.0-76.3%) and historical (70.6-99.3%) controls; (ii) partially ossified occipital in all treated groups (95.8-100%) compared to concurrent (82.5%) and historical (0.0-38.8%) controls; (iii) not ossified odontoid in all treated groups (17.5-56.1%) compared to concurrent (8.8%) and historical (4.3-13.1%) controls; (iv) unilateral cervical rib in the 50 and 125 mg/kg/day fetuses (9.5-18.4%) compared to concurrent (2.5%) and historical (4.0-12.9%) controls; (v) bilateral cervical rib in the 50 and 125 mg/kg/day fetuses (6.0-17.5%) compared to concurrent (0.6%) and historical (1.1-3.5%) controls; (vi) unilateral or bilateral cervical rib in the 50 and 125 mg/kg/day fetuses (15.5-36.0%) compared to concurrent (3.1%) and historical (5.7-14.0%) controls; (vii) bilateral extra (14) ribs in the 50 and 125 mg/kg/day fetuses (37.5-71.1%) compared to concurrent (11.9%) and historical (3.6-24.8%) controls; (viii) unilateral or bilateral extra (14) ribs in the 50 and 125 mg/kg/day fetuses (54.2-86.8%) compared to concurrent (21.9%) and historical (7.9-36.3%) controls; (ix) partially ossified parietals at 125 mg/kg/day (7.9%) compared to concurrent controls (2.5%); (x) increased fontanelle at 125 mg/kg/day (14.0%) compared to concurrent (0%) and historical (0.0-7.6%) controls; (xi) partially ossified cervical arches between and including #3 and #6 at 125 mg/kg/day (6.1%) compared to concurrent (0%) and historical (0%) controls; (xii) partially ossified 1st sternebra at 125 mg/kg/day (10.5%) compared to concurrent controls (1.9%); (xiii) partially ossified 2nd sternebra at 125 mg/kg/day (21.1%) compared to concurrent controls (4.4%); (xiv) not ossified 5th sternebra at 125 mg/kg/day (6.1%) compared to concurrent controls (0.6%); (xv) partially ossified 6th sternebra at 125 mg/kg/day (9.6%) compared to concurrent controls (1.3%); (xvi) not ossified 6th sternebra at 125 mg/kg/day (7.9%) compared to concurrent controls (0%); and (xvii) partially ossified frontals at 125 mg/kg/day (8.8%) compared to concurrent controls (0.6).

TABLE 7a. Selected skeletal findings (% fetuses affected) [a]

Observation	Dose (mg/kg/day)				
	0	10	50	125	Historical controls [b]
Malformations (major defects)					
Kinky ribs	---	---	---	1.8	NA
Multiple defect [c]	---	---	0.6	---	NA
Variations (minor defects)					
No. Fetuses (litters) examined	160 (20)	166 (21)	168 (23)	114 (21)	NA
Calcaneum/Calcanea – Not ossified					
(bilateral)	70.0	82.5**	93.5**	100**	78.9-86.9
(unilateral or bilateral)	76.3	86.7*	95.2**	100**	70.6-99.3
Occipital – Partially ossified	82.5	95.8**	98.8**	100**	0.0-38.8
Odontoid – Not ossified	8.8	17.5*	25.0**	56.1**	4.3-13.1
Cervical rib (unilateral)	2.5	3.6	9.5**	18.4**	4.0-12.9
(bilateral)	0.6	1.8	6.0**	17.5**	1.1-3.5
(unilateral or bilateral)	3.1	5.4	15.5**	36.0**	5.7-14.0
Extra (14) ribs (bilateral)	11.9	10.2	37.5**	71.1**	3.6-24.8
(unilateral or bilateral)	21.9	24.7	54.2**	86.8**	7.9-36.3
Parietals – Partially ossified	2.5	1.2	0.6	7.9*	NA
Fontanelle – Increased	---	1.2	1.8	14.0**	0.0-7.6
Cervical arches between and including 3 and 6 partially ossified	---	---	---	6.1**	NA
Sternebrae					
1st sternebra – Partially ossified	1.9	1.8	2.4	10.5**	NA
2nd sternebra – Partially ossified	4.4	6.6	6.0	21.1**	NA
5th sternebra – Not ossified	0.6	1.8	1.2	6.1*	NA
6th sternebra – Partially ossified	1.3	1.2	1.2	9.6**	NA
Not ossified	---	---	0.6	7.9**	NA
Frontals – Partially ossified	0.6	---	0.6	8.8**	NA

a Data were obtained from Tables 7 and 8 on pages 31 and 33-37 of the study report.
b Historical control data were obtained from pages 52-57 of the study report.
c Multiple defects were noted in a single fetus at 50 mg/kg/day. This fetus had 7th cervical vertebral arches missing, 4th cervical arch missing on the right side, all cervical centra present but misaligned, 5th thoracic arch and 5th rib on the right side missing, and 3rd and 4th thoracic arches slightly misaligned.
--- No animals affected (i.e., zero incidence)
NA Not available
* Significantly different from the controls at $p \leq 0.05$
** Significantly different from the controls at $p \leq 0.01$

Mean scores for ossification of the bones of the hand (*manus*) and foot (*pes*) are included in Table 7b. Mean scores for ossification of the *manus* were increased ($p \leq 0.05$) in all treated groups (2.66-3.13) compared to concurrent (2.42) and historical (1.88-2.59) controls. Similarly, mean scores for ossification of the *pes* were increased ($p \leq 0.01$) in all treated groups (3.06-3.63) compared to concurrent (2.72) and historical (2.53-3.05) controls. These increased scores indicate a dose-dependent increase in partial and/or non ossification of the metacarpals/metatarsals and phalanges. No other skeletal findings could be attributed to treatment.

TABLE 7b. Mean score for skeletal ossification of the hand (*manus*) and feet (*pes*) [a]					
Interval	Dose (mg/kg/day)				
	0	10	50	125	Historical controls [b]
Number of litters examined	20	21	23	21	
Mean *manus* score	2.42	2.66*	2.65*	3.13**	1.88-2.59
Mean *pes* score	2.72	3.06**	3.21**	3.63**	2.53-3.05

a Data were obtained from Table 9 on page 38 of the study report. Scale for semi-quantitative assessment of skeletal ossification of the *manus* and *pes* was obtained from Appendix 4 on page 45 of the study report (1 = good; 4 = poor), included as an Attachment to this DER. Standard deviations were not provided.

b Historical control data were obtained from page 58 of the study report.

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the control group at $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** It was concluded that the maternal LOAEL was 125 mg/kg/day based on decreased body weight gain and food consumption and staining of the fur in the genital area. The maternal NOAEL was 50 mg/kg/day. Additionally at 125 mg/kg/day, decreased fetal body weight and an increase in embryo and fetal lethality were observed, resulting in reduced litter size. Skeletal ossification was dose-dependently decreased in the fetuses in all treated groups. In the 10 and 50 mg/kg/day groups, only specific areas of reduced ossification were evident. At 10 mg/kg/day, the specific bones affected (i.e., the odontoid and calcanea) are often incompletely ossified at GD 21; the slight reduction in fetal body weight compared to controls may have contributed to this effect. However, an effect on ossification of the overall skeleton was apparent at 125 mg/kg/day, and this was likely a direct consequence of maternal toxicity. An increased incidence in the number of fetuses with extra ribs (either cervical or thoracic) was observed at 50 and 125 mg/kg/day. Although the biological significance of this finding is uncertain, it is generally regarded as indicative of fetotoxicity rather than teratogenicity. Thus, it was concluded that the developmental LOAEL was 50 mg/kg/day based on fetotoxicity (extra ribs) and skeletal retardation, and the developmental NOAEL was 10 mg/kg/day.

B. **REVIEWER COMMENTS**

1. **Maternal toxicity:** All dams survived until scheduled termination. There were no treatment-related macroscopic findings.

   Increased incidence of staining of the genital/ventral fur was observed primarily during the dosing period in 16 dams at 125 mg/kg/day compared to 7 dams in the control group. Additionally at 125 mg/kg/day, maternal body weight gains were decreased ($p \leq 0.01$) during the treatment and post-treatment intervals, and for the overall study. Overall net weight gain, corrected for gravid uterine weight, was decreased compared to controls. Food consumption was decreased at this dose compared to controls during the treatment and post-treatment intervals.

The maternal LOAEL is 125 mg/kg bw/day based on increased incidence of ventral/genital staining of the fur and decreased maternal body weight gains and food consumption. The maternal NOAEL is 50 mg/kg bw/day.

2. **Developmental toxicity**

a. **Deaths/resorptions:** The number and percent of early intrauterine deaths were increased at 125 mg/kg/day compared to controls, with a significantly higher ($p \leq 0.05$) proportion of dams affected. Similarly, the number and percent of late intrauterine deaths were increased at 125 mg/kg/day, with a significantly higher ($p \leq 0.05$) proportion of dams affected. The increases in early and late intrauterine deaths were reflected by an increased post-implantation loss at this dose.

The number of dams with live fetuses was reported, but no explanation was provided for the difference between the number of females mated and the number of females with live fetuses. Thus, the reviewers were unable to determine if the number of animals without live fetuses in each group was due to non-pregnancy, abortion, premature delivery, or complete litter resorption. Because no mention was made of abortion, premature delivery, or complete litter resorption, it is likely that these animals were not pregnant. However, the number of females with live litters was comparable among treated and control groups. The number of live fetuses/dam was decreased (↓31) in the 125 mg/kg/day group compared to controls.

b. **Altered growth:** At 125 mg/kg/day, decreases ($p \leq 0.01$) in mean gravid uterine weight, total litter weight, and live fetal body weights were observed.

Incidences of the following skeletal variations, indicating skeletal retardation, were increased ($p \leq 0.05$) over concurrent controls and/or the provided historical control data: (i) in all treated groups - incompletely ossified unilateral and/or bilateral calcanea, partially ossified occipital, and not ossified odontoid; (ii) in the 50 and 125 mg/kg/day fetuses - unilateral and/or bilateral cervical rib and unilateral and/or bilateral extra (14) ribs; (iii) at 125 mg/kg/day - partially ossified parietals, increased fontanelle, partially ossified cervical arches between and including #3 and #6, partially ossified 1st sternebra, partially ossified 2nd sternebra, not ossified 5th sternebra, partially ossified, not ossified 6th sternebra, and partially ossified frontals.

Mean scores for ossification of the *manus* were marginally increased in all treated groups (2.66-3.13) compared to concurrent (2.42) and historical (1.88-2.59) controls. Similarly, mean scores for ossification of the *pes* were also marginally increased in all treated groups (3.06-3.63) compared to concurrent (2.72) and historical (2.53-3.05) controls. These changes could be interpreted as part of the colony dynamics in which the entire pool from which the animals were obtained exhibited higher background incidences of increased ossification of the *manus* and *pes*.

c. **Developmental variations:** Aside from the variations listed above indicating skeletal

retardation, there were no treatment-related external, visceral, or skeletal variations.

d. **Malformations:** There were no treatment-related external, visceral, or skeletal malformations.

The rationale for setting the NOAEL/LOAEL at 10/50 mg/kg/day is that at 10 mg/kg/day: 1) there were no treatment-related external, visceral or skeletal variations; 2) the partial skeletal ossification effects were considered to be isolated effects; there were no other effects; 3) the skeletal variations were minimal in terms of adversity; the effects did not appear to have a steep dose response; and in consideration of mean *manus* and *pes* scores, they did not vary much outside of the historical control range; 4) these effects were not reproduced in the developmental rabbit study at doses up to 15 mg/kg/day; 5) while there appeared to be a quantitative susceptibility because maternal effects occurred at a higher dose (decrease body weight/food consumption at LOAEL=125 mg/kg/day), the qualitative susceptibility is very slight; when considering partial ossification compared to body weight gain reduction.

The developmental LOAEL is 50 mg/kg bw/day based on delayed ossification or non-ossification of the skeleton in the fetuses. The developmental NOAEL is 10 mg/kg bw/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not affect the acceptability or the conclusions of this DER:

- Dams were treated only during GD 6-15 instead of GD 6-21. However, this dosing duration covered the period of organogenesis, and was considered acceptable according to the guidelines established shortly after the completion of this study (Pesticide Assessment Guideline §82-2, Subdivision F; November, 1984).

- The only summary data provided for clinical observations was ventral/genital staining of the fur.

- Although it was stated that no macroscopic lesions were noted during the maternal necropsies, no summary data were provided.

- The developmental variations/malformations were not presented on a per litter basis.

ATTACHMENT

The following is page 45, Appendix 4 of the study report.

APPENDIX 4

SCALE FOR ASSESSMENT OF SKELETAL OSSIFICATION OF THE MANUS AND PES

Scale

1 (good) Metacarpals/metatarsals and first and third rows of phalanges fully ossified (or one phalanx partially ossified).

2 Metacarpals/metatarsals fully ossified. First or third row of phalanges ossified, although an occasional phalanx (approximately up to four) may be partially ossified.

3 Metacarpals/metatarsals fully or occasionally partially ossified. First row of phalanges either partially or not ossified together with third row of phalanges either partially or fully ossified.

4 (poor) Metacarpals/metatarsals - some either partially or not ossified plus first row of phalanges usually not ossified and third row of phalanges partially ossified.